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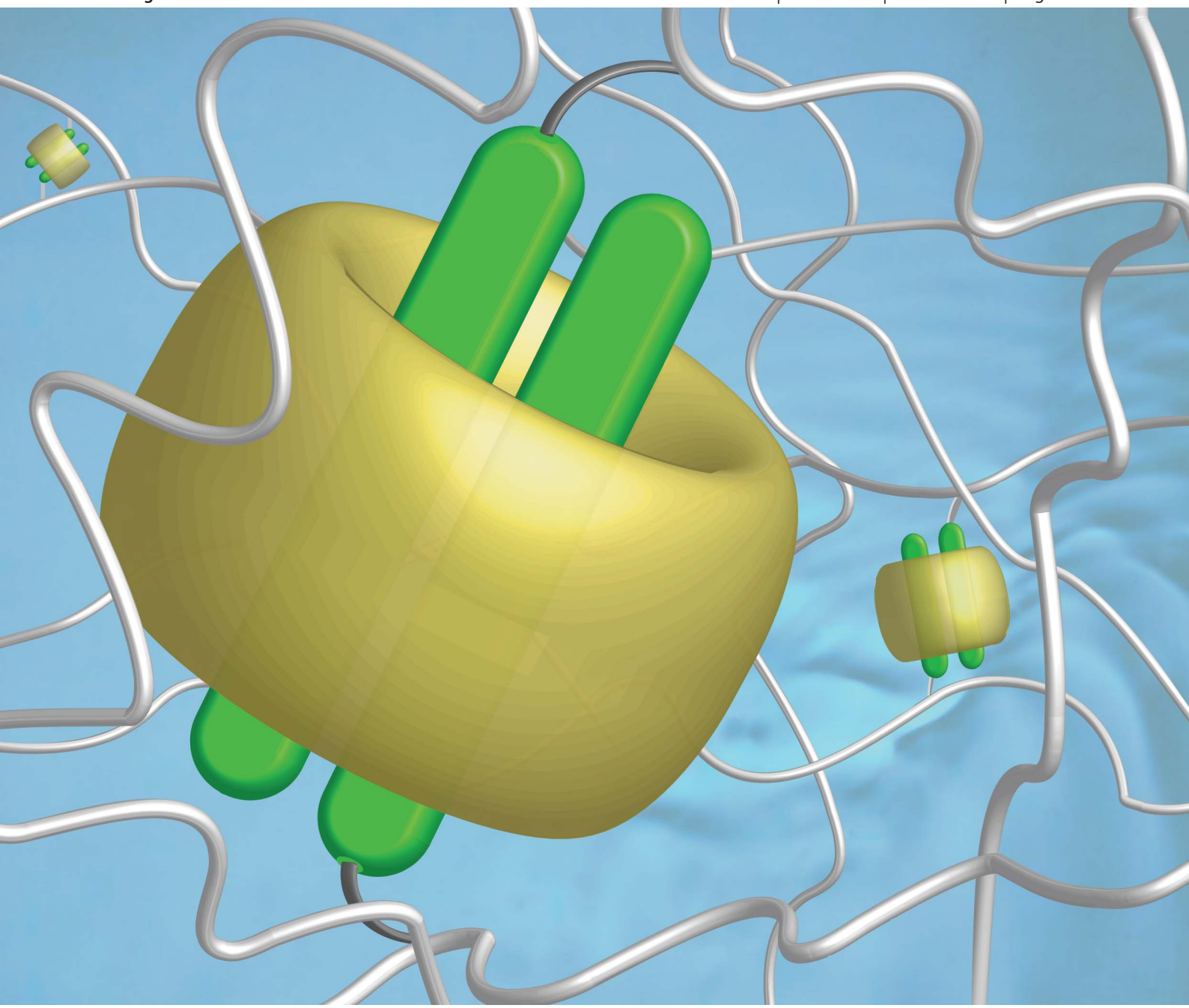

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Dynamically crosslinked materials *via* recognition of amino acids by cucurbit[8]uril†

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Hydrogels, an increasingly important class of material, have physical properties amenable to many potential uses, particularly in the biomedical area. Utilisation of hydrogels, however, relies not only on their mechanical properties but also on a favourable toxicity profile. Self assembly of polymers through naturally occurring and non-toxic units is therefore a very attractive option. The aromatic amino acids phenylalanine and tryptophan are two such molecular units that form 2 : 1 complexes with cucurbit[8]-uril (CB[8]) with high binding equilibrium constants (K_{eq} up to 10^{12} M^{-2}). Herein, water soluble styrenic monomers were copolymerised with synthetically derived aromatic amino acid monomers of phenylalanine and tryptophan. The resulting polymers were shown to form dynamic and self-healing physically crosslinked hydrogels *via* recognition and binding of the amino acids to cucurbit[8]uril.

1 Introduction

Hydrogels are becoming an increasingly important class of material and can be prepared using either covalent or non-covalent approaches.[1–4] However, covalently crosslinked hydrogel materials are often brittle and lack the ability to self heal once the network is broken.[5] These shortcomings have often been addressed by employing dynamic and reversible non-covalent interactions as the structural crosslinks in hydrogels.[6] Cucurbit[*n*]uril (n = 5–8 and 10; CB[*n*]) are a family of macrocyclic host molecules, which offer attractive supramolecular interactions for such applications. These macrocyclic hosts are methylene-linked oligomers of glycoluril that are symmetric and 'barrel'-like in shape with two identical portal regions laced by ureido-carbonyl oxygens. The number of glycoluril units determines the size of the CB[*n*] cavity without affecting the height of the molecular container (approximately 0.9 nm), similar to the cyclodextrin family, and these materials have recently been screened for toxicity demonstrating that they are generally not toxic.[7,8] Smaller homologues of the CB[*n*] family (*i.e.* CB[5], CB[6] and CB[7]) are capable of binding single guests (typically cationic amines, metal, imidazolium ions or small molecule drugs).[9–12] In contrast to the smaller CB[*n*] homologues, CB[8] has a larger cavity volume (479 $Å^3$)[13,14] capable of simultaneously accommodating two planar and hydrophobic guests in a π–π-stack geometry.[11,15–20] This host has been used most prominently in a 1 : 1 : 1 'hetero'-ternary complex using an electron-deficient first guest, such as methyl viologen (MV), and an electron-rich second guest such as naphthol, pyrene and dibenzylfuran.[18,20–22] In favourable cases, exceptionally high overall equilibrium binding affinities (K_{eq} (overall) = K_{eq} (1) × K_{eq} (2) up to 10^{14} M^{-2}) were reported,[13,18,21,23] leading to utilisation in a number of applications ranging from the formation of diblock copolymers,[24–26] sequence-selective recognition of peptides,[27] self-sorting systems,[28] surface modification,[29,30] protein conjugation,[31] to the formation of nanocapsules,[32] nanocomposites[33] and hydrogels.[34,35]

Our approach involves the use of polymeric materials coupled with strong, reversible, and stimuli-responsive CB[8]-based 2 : 1 'homo'-ternary binding motifs of naturally occurring amino acid derived guests. Urbach and coworkers have demonstrated that *N*-terminally charged aromatic amino acids, such as phenylalanine and tryptophan, bind in a 2 : 1 fashion forming a ternary complex with CB[8] through multiple non-covalent interactions acting synergistically. This results in exceptionally high equilibrium binding affinities (K_{eq} up to 10^{11} M^{-2}).[36] The ternary complex likely forms in a stepwise binding process whereby one amino acid guest enters first (K_{eq1}) followed by the second amino acid guest (K_{eq2}). These differ from previous guests as they (1) do not always yield a visible charge-transfer complex, and (2) the guests should not carry a significant toxicity profile as they are naturally occurring amino acids. This 2 : 1 host–guest system has been exploited previously in a variety of systems including some of biological relevance such as the dimerisation of proteins which could be observed by FRET analysis,[37–40] and also in insulin sensing.[41]

Herein, we utilise this 2 : 1 homoternary binding motif for the preparation of supramolecular hydrogels. By employing commercially available and natural amino acids such as phenylalanine and tryptophan, the hydrogel system should not only display a reduced toxicity profile but is simultaneously

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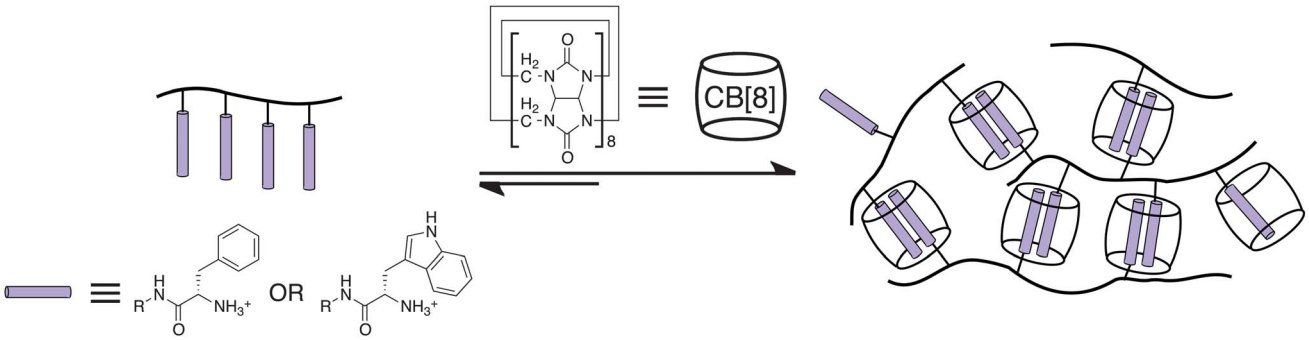

Fig. 1 Schematic of hydrogel formation upon CB[8] addition. The amino acids (represented by the shaded cylinders) bind in a 2 : 1 fashion with CB[8]. The R group of the amino acid is encapsulated within the hydrophobic cavity by non-covalent interactions. Further interactions occur between the protonated *N*-terminus of the amino acid unit and the CB[8] portal carbonyl groups.

simplified from a three component to a two component system (Fig. 1). The well known stimuli-responsive nature of the ternary complex and the ease of synthesis of the various components make this system well suited for a variety of important biomedical and industrial applications.

2 Experimental

2.1 Materials and methods

^{1}H-NMR (500 MHz) spectra was recorded using a Bruker Avance 500 Cryo Ultrashield. Chemical shifts are measured in ppm (δ) in $CDCl_3$ and D_2O with the internal references set to δ 7.26 ppm and 4.79 ppm, respectively. ^{13}C-NMR (125 MHz) spectra were recorded in $CDCl_3$ with an internal reference set to $\delta = 77.16$ ppm. ATR FT-IR spectroscopy was performed using a PerkinElmer Spectrum 100 series FT-IR spectrometer equipped with a universal ATR sampling accessory. High resolution mass spectrometry was recorded using a Waters LCT ESI. Gel permeation chromatography (GPC) was carried out in water (H_2O) on Jordi divinylbenzene columns with a Shimadzu SPD-M20A prominence diode array detector, an Optilab refractive index detector and a dynamic light scattering detector (both Wyatt). Samples were filtered over 0.2 μm PVDF filters before injection using a 0.6 ml min^{-1} flow rate. Rheological characterisation was performed using a TA Instruments DHR-2 controlled stress rheometer fitted with a peltier stage set to 20 °C. Dynamic oscillatory strain amplitude sweep measurements were conducted at a frequency of 10 rad s^{-1}. Dynamic oscillatory frequency sweep measurements were conducted at a 10–50% strain amplitude depending on the material viscosity. All measurements were performed using a 40 mm parallel plate geometry with a gap of 0.500 mm and analysed using TA Instruments TRIOS software. Cucurbit[8]uril was prepared according to literature procedures.[16] (4-Vinylbenzyl) amine was purchased from TCI, all other materials purchased from Aldrich and used as received. Dialysis of the functionalised polymers was carried out by dissolving the polymer (on the order of 2 g) in pure water (100 ml) and then placing the solution into a dialysis tube (Spectrum Labs, Spectra/Por, standard grade regenerated cellulose dialysis membrane 6, MWCO 2000 Daltons), which was subsequently submerged into 3 l of water. The external water was then stirred at room temperature and replaced periodically over a 24 h time period (*ca.* 4–5 times). The dialysed polymer solution was then transferred into a round bottom flask, frozen with liquid nitrogen and then lyophilised overnight to yield fluffy white solid materials.

2.2 Synthetic procedures

2.2.1 Synthesis of *tert*-butyl(1-oxo-3-phenyl-1-((4-vinylbenzyl)amino)propan-2-yl)carbamate (StPhe). (4-Vinylbenzyl)-amine (0.500 g, 3.75 mmol) was added dropwise to a solution of Boc–Phe–OSu (1.770 g, 4.88 mmol) in dichloromethane (DCM, 10 ml) at 0 °C. Triethylamine (0.988 ml, 7.50 mmol) was then added dropwise to the cooled solution and the reaction was allowed to warm to room temperature and stirred for 24 hours. The reaction was quenched with saturated sodium carbonate solution and the product extracted with dichloromethane (DCM) (×3). The combined organic extracts were dried with magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was redissolved in a 50 : 50 mixture of hexane and ethyl acetate and washed through a pad of silica. Removal of the solvent *in vacuo* yielded the title compound as an amorphous white solid which was dried under a high vacuum overnight (1.324 g, 93%). ^{1}H-NMR Spectroscopy ($CDCl_3$, 500 MHz) δ (ppm) = 7.35–6.95 (9H, m, Ar–*H*), 6.73–6.63 (1H, dd, $J = 17.5$ Hz, 10.7 Hz, alkene–*H*), 6.10–6.02 (1H, br s, N–*H*), 5.78–5.68 (1H, dd, $J = 17.5$ Hz, 0.8 Hz, alkene–*H*), 5.28–5.20 (1H, dd, $J = 10.7$ Hz, 0.8 Hz, alkene–*H*), 5.10–4.95 (1H, br s, N–*H*), 4.40–4.37 (3H, m, C*H*$_2$, C*H*), 3.16–2.99 (2H, m, C*H*$_2$), 1.39 (9H, s, C*H*$_3$). ^{13}C-NMR Spectroscopy ($CDCl_3$, 125 MHz) δ (ppm) = 170.98 (*C*O), 155.37 (*C*O), 137.19 (Ar*C*), 136.87 (Ar*C*), 136.63 (Ar*C*), 136.32 (*C*H), 129.33 (Ar*C*H), 128.72 (Ar*C*H), 127.86 (Ar*C*H), 126.95(Ar*C*H), 126.41 (Ar*C*H), 113.95 (*C*H$_2$), 56.08 (*C*H), 43.19 (*C*H$_2$), 38.53 (*C*H$_2$), 28.24 (*C*H$_3$). Elemental: found C, 72.39; H, 7.51; N, 7.22%. $C_{23}H_{26}O_3N_2$ calculated C, 72.60; H, 7.42; N, 7.36. FT-IR (ATR) $\nu = 3339$ (m), 2983 (m), 1678 (s), 1658 (s), 1517 (s) cm^{-1}. HRMS: found 381.2178 $[C_{23}H_{26}O_3N_2]^+$ calculated 381.2190.

2.2.2 Synthesis of poly(2-amino-3-phenyl-*N*-(4-vinylbenzyl)-propanamide-*co*-(vinylbenzyl)trimethylammonium chloride) (StPhe-StAm). StPhe (0.498 g, 1.31 mmol), (vinylbenzyl)trimethylammonium chloride (2.500 g, 11.81 mmol) and 4,4′-azobis(4-cyanopentanoic acid) (ACPA, 36.4 mg) were dissolved in

methanol (MeOH, 7 ml) and degassed with nitrogen for 30 minutes. The reaction was heated to 70 °C and stirred for 24 hours. The product was precipitated with diethyl ether and collected by vacuum filtration. The crude residue was redissolved in MeOH (20 ml) and 4 N hydrogen chloride in dioxane solution (20 ml) added dropwise. The reaction was stirred for 8 hours and the deprotected polymer was precipitated with diethyl ether. The crude residue was purified by dialysis against water over 24 hours and the product lyophilised to yield an amorphous white solid (1.774 g, 62%) that was 7% StPhe and 93% StAm. ^{1}H-NMR Spectroscopy (D_2O, 500 MHz) see Fig. S1.† FT-IR (ATR) ν = 3374 (br), 3014 (m), 2923 (m), 1680 (m), 1614 (m), 1479 (s) cm^{-1}. GPC (H_2O): M_n = 10.9 kDa, PDI = 2.2.

2.2.3 Synthesis of *tert*-butyl(3-(1*H*-indol-3-yl)-1-oxo-1-((4-vinylbenzyl)amino)propan-2-yl)carbamate (StTrp). (4-Vinylbenzyl) amine (0.500 g, 3.75 mmol) was added dropwise to a solution of Boc–Phe–OSu (1.960 g, 4.88 mmol) in dichloromethane (DCM, 10 ml) at 0 °C. Triethylamine (0.988 ml, 7.50 mmol) was then added dropwise to the cooled solution and the reaction was allowed to warm to room temperature and stirred for 24 hours. The reaction was quenched with saturated sodium carbonate solution and the product extracted with DCM (×3). The combined organic extracts were dried with magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was redissolved in a 50 : 50 mixture of hexane and ethyl acetate and washed through a pad of silica. Removal of the solvent *in vacuo* yielded the title compound as an amorphous white solid which was dried under a high vacuum overnight (1.541 g, 98%). ^{1}H-NMR Spectroscopy ($CDCl_3$, 500 MHz) δ (ppm) = 8.07 (1H, s, Ar–*H*), 7.70–7.62 (1H, d, J = 7.8 Hz, Ar–*H*), 7.39–7.31 (1H, d, J = 8.1 Hz, Ar–*H*), 7.30–7.20 (1H, m, Ar–*H*), 7.20–7.15 (1H, ddd, J = 8.1, 6.9, 1.1 Hz, Ar–*H*), 7.15–7.10 (1H, ddd, J = 8.1, 6.9, 1.1 Hz, Ar–*H*), 7.00–6.85 (3H, m, Ar–*H*), 6.71–6.61 (1H, dd, J = 17.7, 10.9 Hz), 6.08–5.90 (1H, br s, N–*H*), 5.73–5.65 (1H, dd, J = 17.7, 0.8 Hz, alkene–*H*), 5.27–5.20 (1H, dd, J = 10.9, 0.8 Hz, alkene–*H*), 5.27–5.10 (1H, br s, N–*H*), 4.51–4.37 (1H, br s, C*H*), 4.32–4.18 (2H, m, CH_2), 3.39–3.25 (1H, dd, J = 14.3, 5.2 Hz, HC–*H*), 3.25–3.10 (1H, dd, J = 14.3, 7.5 Hz, HC*H*), 1.41 (9H, s). ^{13}C-NMR Spectroscopy ($CDCl_3$, 125 MHz) δ (ppm) = 171.52 (*C*O), 155.45 (*C*O), 136.74 (Ar*C*), 136.20 (*C*H$_2$), 136.05(Ar*C*), 128.02 (A*C*H), 127.82 (Ar*C*), 127.37 (Ar*C*H), 126.32 (Ar*C*H), 123.19 (Ar*C*H), 123.03 (Ar*C*), 122.35 (Ar*C*H), 119.86 (Ar*C*H), 113.92 (*C*H), 111.18 (Ar*C*H), 110.67 (Ar*C*), 55.31 (*C*H), 43.21 (*C*H$_2$), 28.43 (*C*H$_2$), 28.27 (*C*H$_3$). Elemental: found C, 69.27; H, 6.98; N, 9.44%. $C_{25}H_{29}O_3N_3$ calculated C, 71.57; H, 6.97; N, 10.02. FT-IR (ATR) ν = 3310 (br), 2978 (m), 2930 (m), 1693 (s), 1655 (s), 1494 (s) cm^{-1}. HRMS: found 420.2303 $[C_{25}H_{30}O_3N_3]^+$ calculated 420.2287.

2.2.4 Synthesis of poly(2-amino-3-(3*H*-indol-3-yl)-*N*-(4-vinylbenzyl)propanamide-*co*-(vinylbenzyl)trimethylammonium chloride) (StTrp–StAm). StTrp (0.550 g, 1.31 mmol), (vinylbenzyl)trimethylammonium chloride (2.500 g, 11.81 mmol) and ACPA (36.4 mg) were dissolved in MeOH (7 ml) and degassed with nitrogen for 30 minutes. The reaction was heated to 70 °C and stirred for 24 hours. The product was precipitated with diethyl ether and collected by vacuum filtration. The crude residue was redissolved in MeOH (20 ml) and 4 N hydrogen chloride in dioxane solution (20 ml) added dropwise. The reaction was stirred for 8 hours and the deprotected polymer was precipitated with diethyl ether. The crude residue was purified by dialysis against water over 24 hours and the product lyophilised to yield an amorphous white solid (1.752 g, 60%). ^{1}H-NMR Spectroscopy (D_2O, 500 MHz) see Fig. S2.† FT-IR (ATR) ν = 3373 (br), 3012 (br), 2921 (br), 1679 (m), 1614 (m), 1478 (s) cm^{-1}. GPC (H_2O): M_n = 12.1 kDa, PDI = 2.4.

2.3 Hydrogel preparation

Polymer solutions (20% w/v) were prepared and diluted with equivalent volumes of aqueous CB[8] solutions of various concentrations resulting in a final polymer concentration of 10% w/v. The combined solutions were mildly heated and shaken (or preferably agitated with a vortex) for a few seconds before allowing to cool to room temperature so the hydrogel could set.

3 Results

3.1 Design and synthesis of functional building blocks

(Vinylbenzyl)trimethylammonium chloride derived polymers are rigid and highly water soluble on account of their cationic charge, making this monomer ideal for copolymerisation with guest-functional monomers. Polymer rigidity is particularly important for this system in order to enhance hydrogel strength by limiting intramolecular binding of the amino acid units on the same polymer chain. Rigid polymers are ideal as they promote intermolecular complex formation, leading to stronger materials.

For the purpose of copolymerisation, a compatible amino acid monomer was also required to ensure random distribution of the functional units. Therefore, synthesis of a styrene derived amino acid monomer was undertaken, as shown in Fig. 2. Coupling of the activated amino acids Boc-L-phenylalanine *N*-hydroxysuccinimide ester and Boc-L-tryptophan *N*-hydroxysuccinimide ester with (4-vinylbenzyl) amine in the presence of triethylamine afforded the Boc-protected amino acid monomers (StPhe, **3a** and StTrp, **3b**) in good yields.

With the StPhe and StTrp monomers in hand, 'traditional' free-radical copolymerisation with (vinylbenzyl)trimethylammonium chloride was performed using 4,4′-azobis(4-cyanopentanoic acid) (ACPA) (Fig. 3). Following acid treatment for

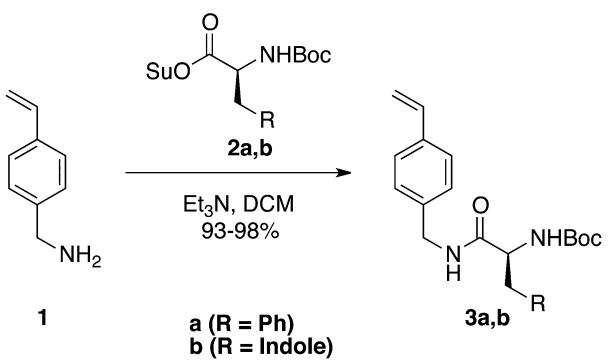

Fig. 2 Synthesis of StPhe (**3a**) and StTrp (**3b**) monomers.

Boc-deprotection, the cationic styrenic amino acid random copolymers (**5a**, StPhe-StAm and **5b**, StTrp-StAm) were afforded as HCl salts, which were highly water soluble and easily purified by dialysis. Proton NMR analysis of these copolymers determined that 7% of the monomeric repeat units were functional amino acids in both the phenylalanine and tryptophan cases (see ESI†). By comparing the integration of the aromatic signal (7.5–6.0 ppm) with the integration of the trimethylammonium singlet (2.7 ppm), the excess of aromatic protons in the polymer was determined, which directly correlated to the number of guest functional monomers incorporated into the final polymers (Fig. S1 and S2†).

3.2 Hydrogel formation

Phenylalanine and tryptophan bind within the CB[8] cavity in a 2 : 1 fashion.[36] Therefore, 0.5 equivalents of CB[8] theoretically affords 100% crosslinking of the aromatic amino acid units pendant from the copolymer backbone. Initial CB[8] titrations into aqueous polymer solutions exemplified this well. Polymer solutions containing 20% by weight of the functional polymer were diluted with equivalent volumes of solutions containing various equivalents of CB[8], mildly warmed and shaken. As hypothesised, addition of CB[8] resulted in large increases in viscosity, which was easily visualised by inverted vial tests (Fig. 4). It was observed that with no CB[8] the polymer solution remained transparent and the colourless solution flowed readily. With 0.35 equivalents of CB[8] the solution became much more viscous but retained some flow. Addition of ≥0.5 equivalents of CB[8] caused the solution flow to slow dramatically upon vial inversion. Addition of 1 equivalent of CB[8] could in theory favour 100% formation of 1 : 1 amino acid⊂CB[8] complexes. As strong hydrogels are still formed with 1 equivalent of CB[8] (Fig. 4) this is clearly not the case.

CB[7] is large enough to bind only one Phe or Trp unit and unable to promote crosslinking, thus, as a control, 0.5 equivalents of CB[7] were also added to an aqueous polymer solution. With CB[7] addition no change in viscosity was observed, therefore 1 : 1 binding of Phe or Trp to a CB[*n*] molecule is not constructive for gel formation. As a second control, a homopolymer, poly[(vinylbenzyl)trimethylammonium chloride] (p-StAm), was also synthesised. Upon addition of CB[8] to an aqueous solution of the control StAm homopolymer at 10% by weight, no visible change in viscosity was observed (Fig. S3†). Hydrogel formation, therefore, arises exclusively from the 2 : 1 binding of the charged amino acids inside the CB[8] cavity and the cationic polymer backbone is not involved in the crosslinking process.

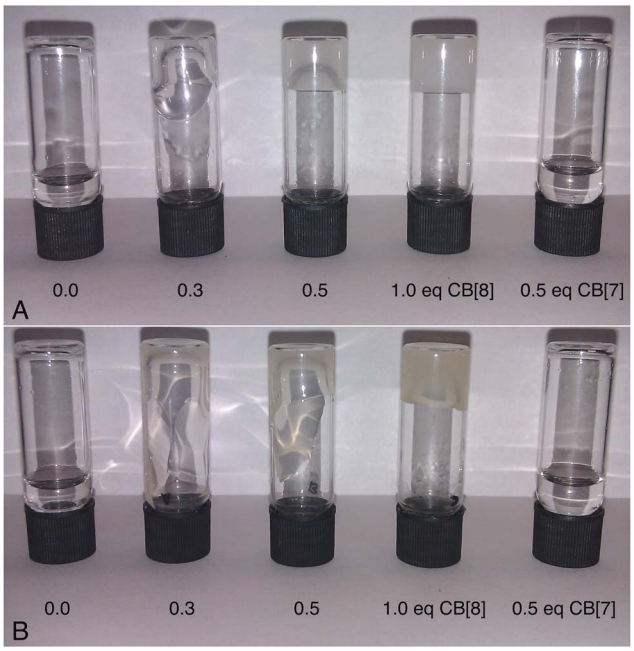

Fig. 4 Inverted vial test of 10% w/v of **5a** (A) or **5b** (B) solutions with varying equivalents of CB[8] and a control experiment wherein addition of CB[7], which can only accommodate one guest (*i.e.* crosslinking is impossible), does not cause a visible increase in viscosity.

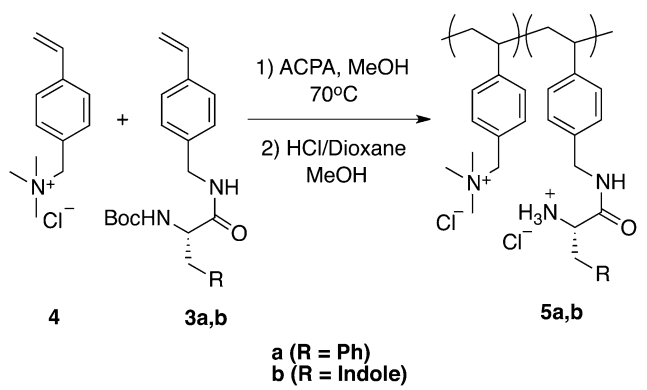

Fig. 3 Two stage synthesis of StAm-StPhe (**5a**) and StAm-StTrp (**5b**) copolymers.

3.3 Rheological characterisation of the supramolecular hydrogels

Supramolecular hydrogels have been successfully designed and prepared and rheological characterisation was performed to quantify their mechanical properties. Rheological analysis was carried out on supramolecular hydrogels prepared from the mixture of the **5a** and **5b** solutions with various molar equivalents of CB[8] (relative to amino acid functionalised units, *e.g.* 0, 0.05, 0.15, 0.35, 0.50, 0.70 equivalents) corresponding to a various theoretical percentage crosslinking. Proceeding beyond the theoretical limit of 100% crosslinking (from 0.5 equivalents of CB[8] to 0.7 equivalents) are two possible outcomes: (a) the gel properties, including viscosity, decrease. In the phenylalanine case 20% of the amino acid units would be bound in a 1 : 1 fashion and only 80% of the amino acid units in a 2 : 1 fashion leading to crosslinking. This would be due to the first binding constant being equivalent to the second. In the tryptophan case, the second binding constant is in fact weaker than the first and so 40% of the amino acids would be bound in a 1 : 1 fashion and 60% in a 2 : 1 fashion.[36] Therefore, at 0.7 equivalents of CB[8] the actual crosslinking would be either 80% or 60% dependant on the amino acids used. (b) As 2 : 1 binding is

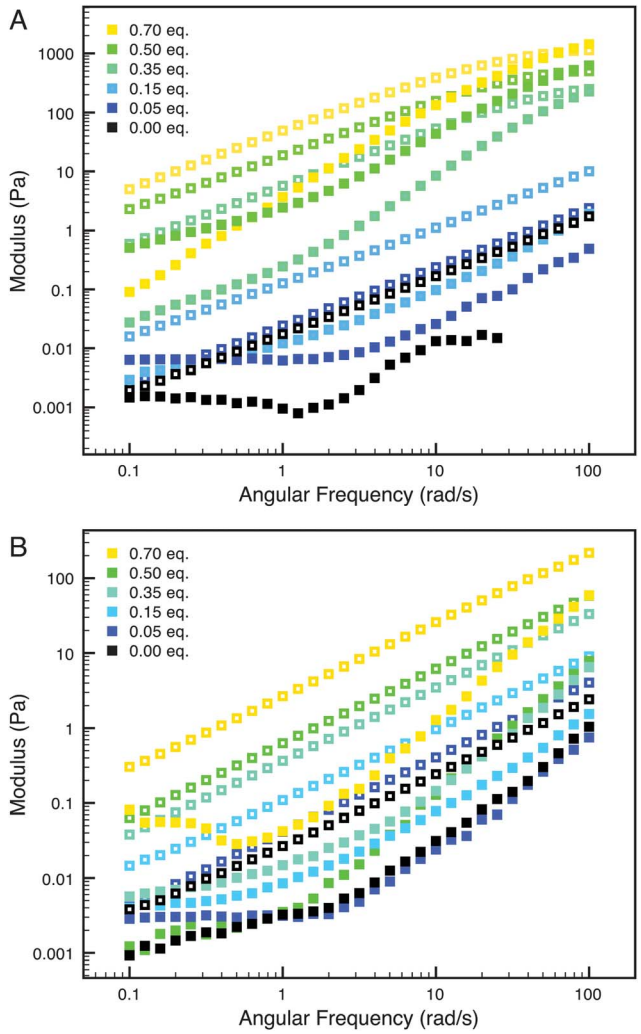

Fig. 5 Frequency-dependent oscillatory rheology of (A) StPhe-StAm, (B) StTrp-StAm hydrogels with varying equivalents of CB[8]. Open symbols are loss moduli G'', and closed symbols are storage moduli G'.

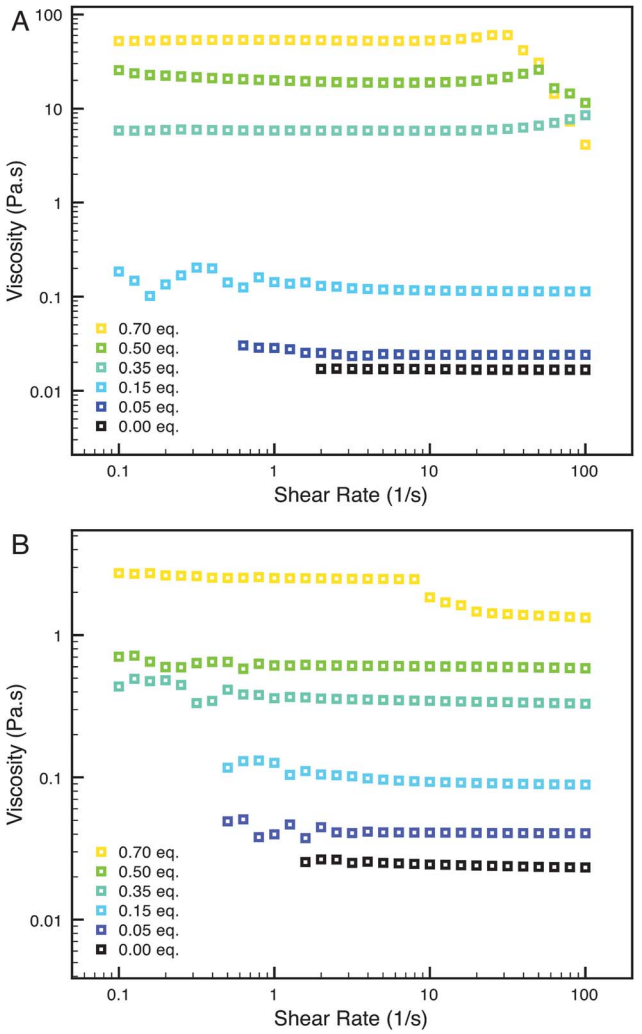

Fig. 6 Steady shear rheological measurements of hydrogels formed with (A) StPhe-StAm (10% w/v), (B) StTrp-StAm (10% w/v) with increasing loading of CB[8].

more favourable than 1 : 1 binding (*i.e.* higher K_{eq} value for the ternary complex in comparison with the binary complex) the excess CB[8] suspended in the solution would not prevent crosslinking as in case 'a' but instead increase viscosity by acting as a solid viscosity modulator. It is also possible that excess CB[8] in solution would promote formation of the ternary complex pushing the system towards 100% crosslinking and reducing the apparent rate of dissociation of the components.

3.3.1 Oscillatory measurements. Strain-dependant oscillatory shear measurements at 20 °C were first performed in order to determine the linear viscoelastic properties of the material. Throughout the experiment all hydrogels were shown to have a broad viscoelastic window and no deviation from linearity was observed even at the highest oscillation strain, except for the StTrp-StAm random copolymer hydrogels containing 0.7 equivalents CB[8] (see ESI Fig. S4 and S5†). Both materials, regardless of CB[8] concentration, retained their broad viscoelastic regions. It was observed that, with increasing CB[8] concentration, the plateau modulus of each material increased, even when CB[8] loading exceeded 0.5 equivalents relative to the guest moieties. Interestingly, the moduli for the StPhe-StAm are distinctly larger than those of the StTrp-StAm hydrogels by an order of magnitude.

Frequency-dependant oscillatory rheological measurements were also performed on the materials within the linear viscoelastic regime (Fig. 5). As with the strain-dependant oscillatory measurements, the moduli of the StPhe-StAm hydrogels are a magnitude higher than those of the StTrp-StAm materials. It is also worthwhile noting that the StPhe-StAm hydrogels become elastically active at lower angular frequencies than the StTrp-StAm hydrogels. In both cases, more equivalents of CB[8] added caused an increase in both the storage and loss moduli of the hydrogel and also a decrease in the angular frequency at which the storage modulus (G') becomes dominant over the loss modulus (G''). With more CB[8] present at any one time a greater number of crosslinks will exist between the polymers, allowing the material to behave more elastically as the supramolecular network undergoes a higher energy penalty to break.

As the crossover point occurs at lower angular frequencies with greater CB[8] concentration it can be inferred that a greater degree of crosslinking has been achieved and that higher CB[8] concentrations (even beyond 0.5 equivalents) enforce 2 : 1 complexation. This leads to the conclusion that the equilibrium of ternary complex formation does not lie completely toward 2 : 1 homoternary complex. Instead we envisage that in such cases the equilibrium between the free amino acid units and CB[8] and their respective 1 : 1 binary complex lies towards the free units. By increasing the CB[8] concentration, 1 : 1 complexation is enforced by Le Chatelier's principle, quickly proceeding to ternary complex formation, which is energetically favourable.

3.3.2 Steady shear measurements. Steady shear rheological measurements were performed on both the StPhe-StAm and StTrp-StAm hydrogels to determine the mechanical effect of varying amounts of CB[8] (Fig. 6). In both cases, the zero-shear viscosity of the materials increased with added equivalents of CB[8], even beyond 0.5 equivalents. Initially, the hydrogels behaved as Newtonian materials with no change in viscosity, however, at high shear rates some hydrogels with higher CB[8] loading displayed a slight degree of shear-thickening as shear rate increased. All materials displayed shear thinning behaviour under high shear conditions.

Considering the zero-shear viscosities alone, upon increasing addition of CB[8] both the StPhe-StAm and StTrp-StAm materials displayed an increase as would be expected (Fig. 7). Whilst initially at low CB[8] concentrations both materials have very low and similar viscosities, once beyond 0.15 equivalents of CB[8], the StPhe-StAm hydrogel zero-shear viscosity increases at a much greater rate. This suggests that the homoternary $Phe_2 \subset CB[8]$ complex is much stronger than the $Trp_2 \subset CB[8]$ complex, consistent with previous observations by Urbach *et al.*[36] Fig. 7 also exemplifies the equivalents of CB[8] required to induce gelation. Extrapolating lines of best fit for each material show gelation points for the two materials to be distinctly different. The StPhe-StAm copolymer requires only 0.31 equivalents of CB[8] for gelation to begin whereas the StTrp-StAm hydrogel requires 0.5 equivalents. For the StTrp-StAm copolymer, viscosity is not dramatically altered with CB[8] concentration until surpassing the theoretical 100% crosslinking point. This is accountable for by the difference in association constants of the amino acids with CB[8] (*vide supra*) and how this may effect the dynamic equilibrium present.

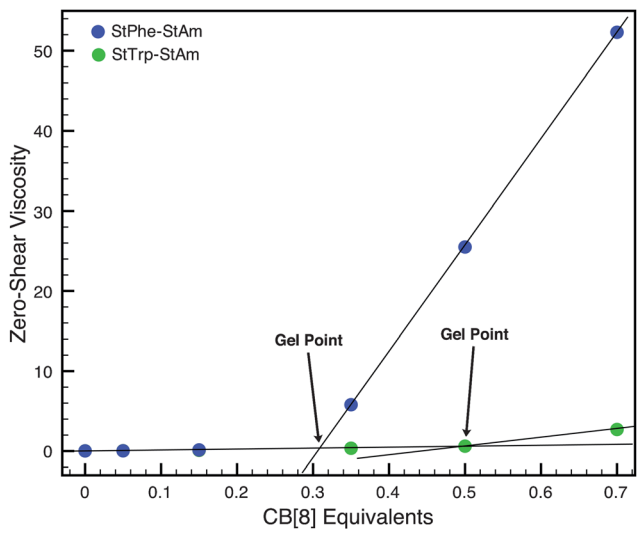

Fig. 7 The effect of CB[8] concentration on zero-shear viscosity at increasing shear rate. Note, six data points were obtained for both systems but the first three are the same.

4 Conclusions

Rigid polymer chains with pendant phenylalanine or tryptophan amino acids have the ability to form hydrogels in the presence of CB[8]. It has been determined that the phenylalanine unit affords much stronger hydrogel materials than its tryptophan counterpart. This work sheds light on improved biomedical systems for drug delivery as hydrogel formation can now be achieved without the requirement of potentially toxic guest moieties for the CB[8]-based crosslinking. Further development of this system will entail attachment of these amino acids to natural polymers such as cellulose derivatives, that will lead to biomedical applications in 3D cell culture, drug delivery and regenerative medicine and would bypass limitations of potentially toxic polymer metabolites. We have demonstrated that the material properties of hydrogels can be readily altered by attenuating the CB[8] concentration as well as by choosing an appropriate amino acid based guest.

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